

Supplement S 1. Measurements of binding kinetics and equilibrium binding for antagonists at room temperature and at 37 °C.

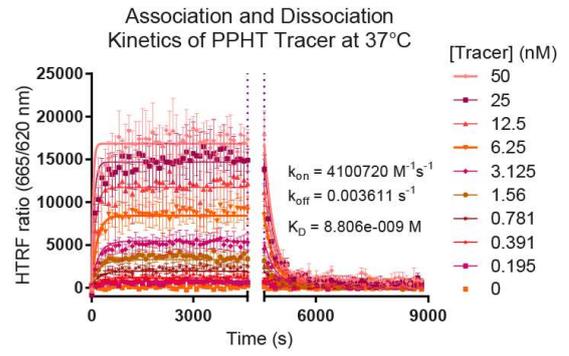
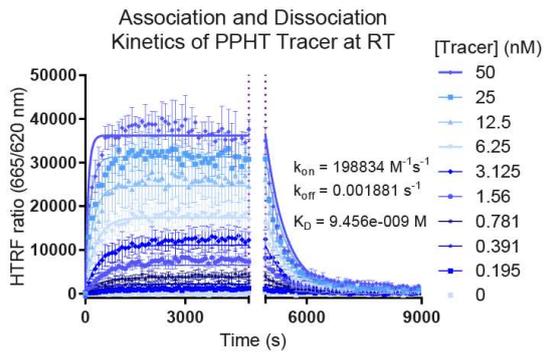
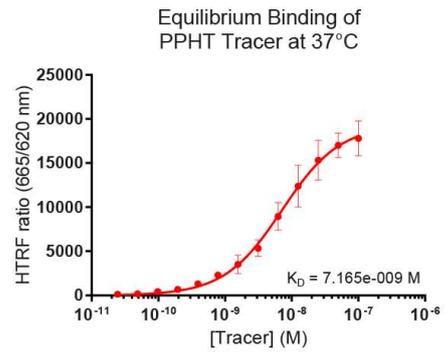
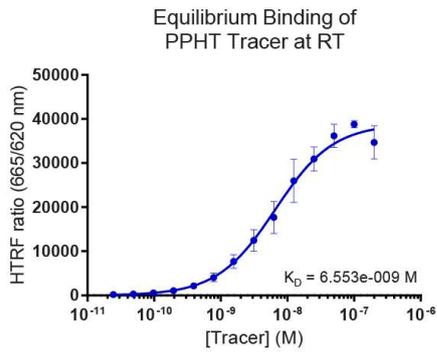
Table S 1. Affinity and kinetic parameters derived from binding equilibrium (ePCA) and binding kinetics (kPCA) measurements. Values represent the mean of two independent experiments with two replicates each (N =2, n =2) at 37°C. NA: only one independent experiment could be evaluated. ND: steady state affinities were beyond the concentration range tested or kinetic traces did not fit to the models used for evaluation.

Compound ID	ePCA		kPCA					
	K _i [M]	SD	K _D [M]	SD	k _{on} [1/(M*s)]	SD	k _{off} [1/s]	SD
(-)-Nemonapride	2,11E-10	5,96E-11	4,96E-11	3,54E-11	1,79E+07	1,95E+06	8,54E-04	5,38E-04
Bromperidol	3,24E-09	1,55E-09	2,52E-09	8,27E-10	2,67E+06	1,04E+06	7,42E-03	2,68E-03
Clozapine	3,12E-08	1,96E-09	5,39E-08	1,33E-08	8,35E+05		2,88E-02	
Domperidone	5,06E-09	8,69E-10	3,15E-09	3,92E-10	6,87E+05	2,62E+05	2,12E-03	5,56E-04
Dopamine	ND	ND	1,07E-06	4,99E-07	1,02E+04	6,02E+03	9,36E-03	1,32E-03
JNJ-37822681	1,61E-08	1,89E-09	1,93E-08	3,99E-09	1,24E+06	9,63E+05	2,19E-02	1,36E-02
JNJ-39269646	7,22E-08	1,18E-08	9,14E-08	1,37E-08	1,71E+05	NA	1,84E-02	NA
Haloperidol	9,48E-10	2,96E-10	3,86E-10	9,95E-11	7,23E+07	NA	3,30E-02	NA
Olanzapine	1,39E-08	1,29E-09	2,17E-08	9,44E-09	1,61E+06	1,00E+06	3,61E-02	5,99E-03
Paliperidone	8,18E-09	1,56E-09	9,25E-09	2,71E-09	1,92E+06	1,56E+06	1,36E-02	5,91E-03
Pimozide	6,62E-10	3,27E-10	5,82E-10	1,40E-10	9,38E+05	5,11E+05	5,10E-04	1,66E-04
Quetiapine	1,63E-07	3,84E-09	1,60E-07	9,72E-08	6,74E+05	4,03E+05	1,03E-01	5,98E-02
Remoxipride	1,23E-07	2,81E-08	1,35E-07	2,86E-08	1,10E+06	4,54E+05	1,30E-01	7,94E-02
Risperidone	1,67E-09	9,11E-10	1,30E-09	7,52E-10	8,41E+06	5,96E+06	1,07E-02	5,02E-03
Sertindole	6,80E-09	1,86E-09	7,52E-09	5,87E-10	1,33E+06	NA	9,45E-03	NA
Spiperone	5,09E-10	2,29E-10	6,58E-11	1,77E-11	2,03E+07	7,02E+06	1,45E-03	6,22E-04
S-(+)-Raclopride	2,19E-09	1,85E-09	1,29E-09	1,18E-10	2,43E+06	8,68E+05	3,08E-03	8,32E-04
Ziprasidone	1,65E-09	2,93E-10	2,28E-09	2,12E-10	3,54E+06	1,38E+06	7,92E-03	2,31E-03

Table S 2. Affinity and kinetic parameters derived from binding equilibrium (ePCA) and binding kinetics (kPCA) measurements. Values represent the mean of two independent experiments with two replicates each (N =2, n =2) at room temperature. NA: only one independent experiment could be evaluated. ND: binding data did not fit to the models used for evaluation.

Compound ID	ePCA		kPCA					
	K _i [M]	SD	K _D [M]	SD	k _{on} [1/(M*s)]	SD	k _{off} [1/s]	SD
(-)-Nemonapride	2,70E-10	5,96E-12	9,58E-11	3,26E-12	5,66E+06	2,73E+05	5,43E-04	4,46E-05
Bromperidol	3,09E-09	3,22E-10	1,89E-09	7,49E-10	2,26E+06	9,57E+05	3,91E-03	1,21E-04
Clozapine	3,42E-08	3,93E-09	5,05E-08	1,28E-08	1,20E+06	1,44E+06	5,13E-02	5,74E-02
Domperidone	4,18E-09	1,08E-09	3,04E-09	5,08E-10	1,81E+05	5,26E+04	5,37E-04	6,75E-05
Dopamine	ND	ND	1,27E-06	5,56E-07	1,88E+04	2,16E+04	2,82E-02	3,01E-02
JNJ-37822681	1,24E-08	2,14E-09	9,32E-09	2,71E-09	7,33E+05	NA	9,54E-03	NA
JNJ-39269646	3,96E-08	2,10E-09	4,87E-08	8,35E-09	4,53E+06	4,51E+06	1,79E-01	1,64E-01
Haloperidol	9,88E-10	1,55E-10	3,82E-10	4,98E-11	1,21E+07	5,18E+06	4,48E-03	1,37E-03
Olanzapine	1,37E-08	9,41E-10	8,58E-09	3,38E-09	> 7.30E+05	NA	> 1.00E-02	NA
Paliperidone	7,39E-09	9,15E-10	5,45E-09	2,07E-09	6,81E+05	1,83E+05	3,52E-03	4,14E-04
Pimozide	1,22E-09	4,23E-10	2,55E-10	6,74E-11	3,10E+05	2,45E+05	7,08E-05	4,17E-05
Quetiapine	1,26E-07	3,48E-08	1,50E-07	6,94E-08	1,03E+05	2,04E+04	1,69E-02	6,07E-03
Remoxipride	7,01E-08	1,08E-08	8,31E-08	3,47E-08	3,28E+05	NA	3,14E-02	NA
Risperidone	1,05E-09	4,07E-10	7,56E-10	7,62E-11	4,43E+06	8,54E+05	3,31E-03	3,09E-04
Sertindole	6,15E-09	3,21E-09	4,07E-09	2,23E-09	7,70E+05	7,00E+05	2,35E-03	1,13E-03
Spiperone	2,96E-10	1,43E-10	1,79E-10	4,11E-12	5,44E+06	1,11E+06	9,70E-04	1,76E-04
S-(+)-Raclopride	1,35E-09	9,97E-10	6,34E-10	1,15E-10	9,57E+05	1,81E+05	5,96E-04	4,62E-06
Ziprasidone	1,76E-09	4,12E-10	1,31E-09	8,40E-11	1,29E+06	2,01E+05	1,67E-03	1,55E-04

Figure S1a



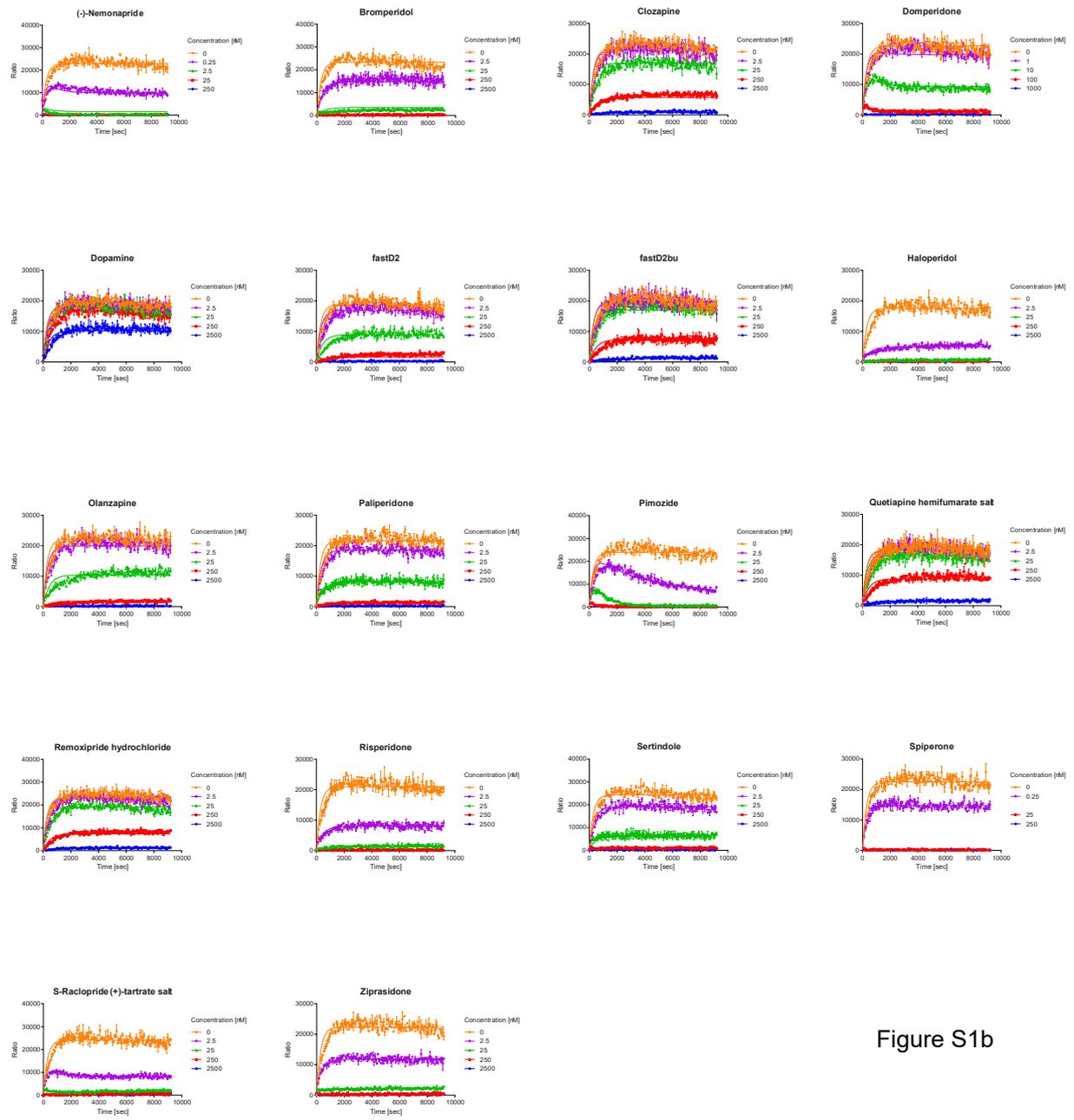


Figure S1b

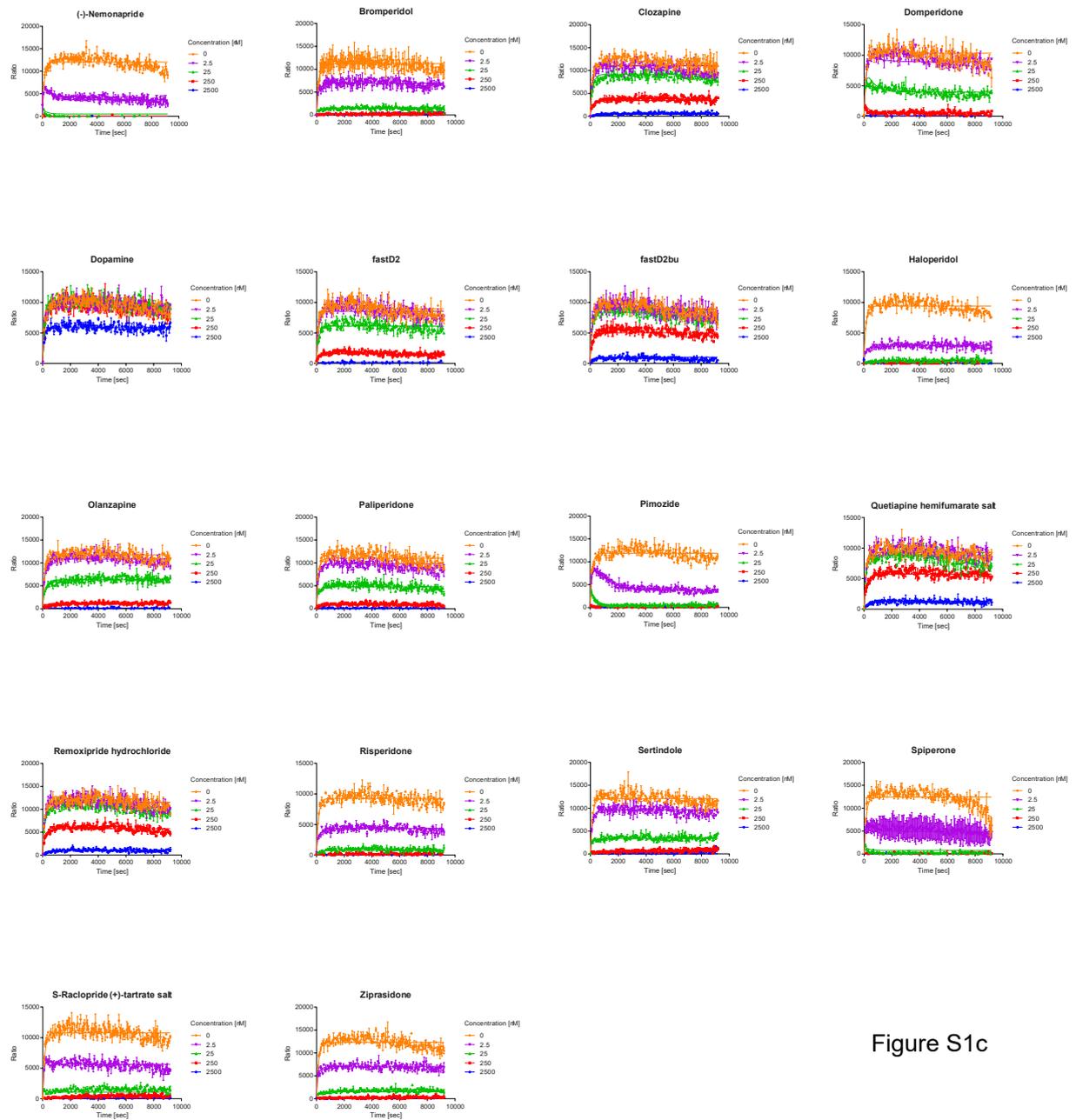


Figure S1c

Figure S1d

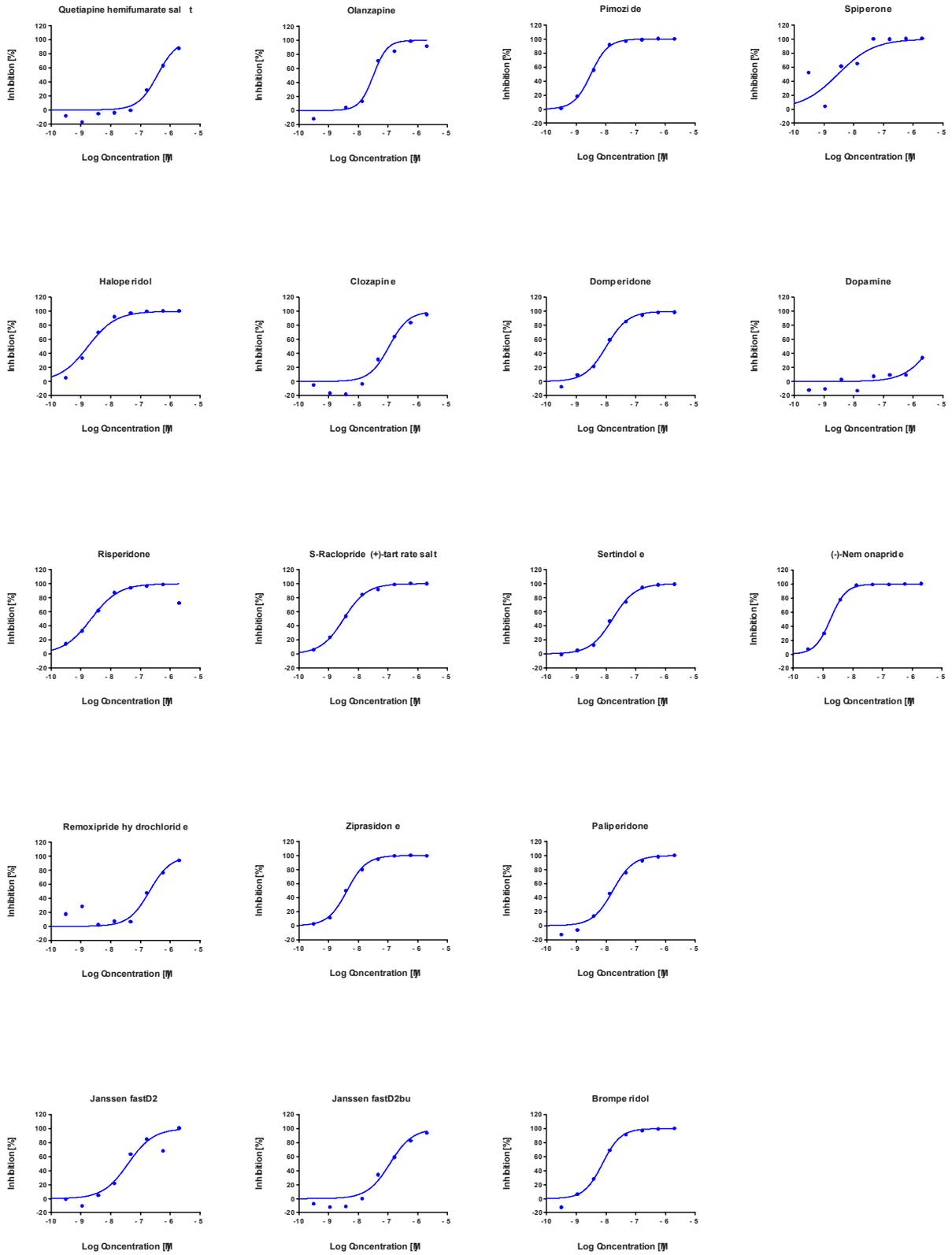


Figure S1e

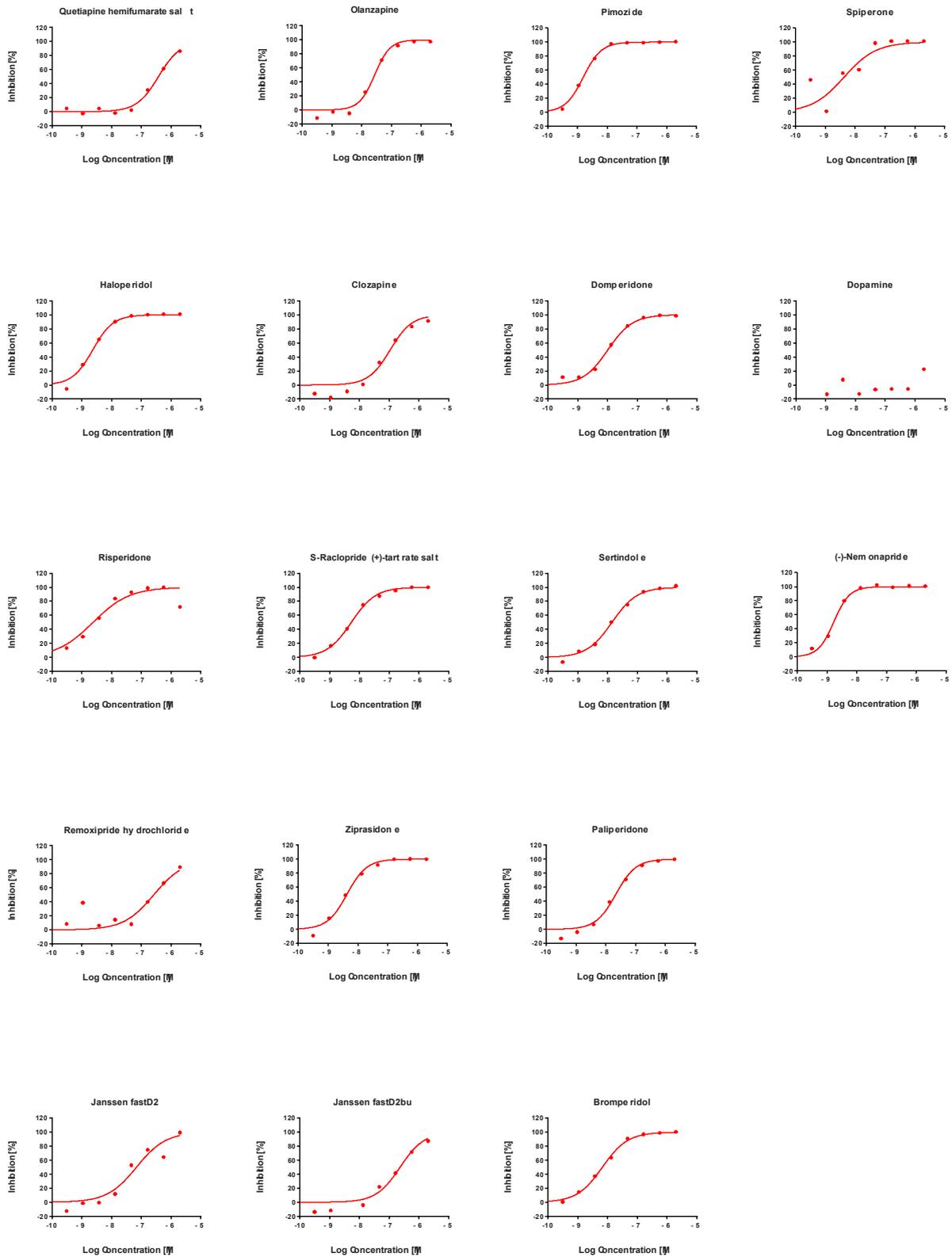


Figure S1f

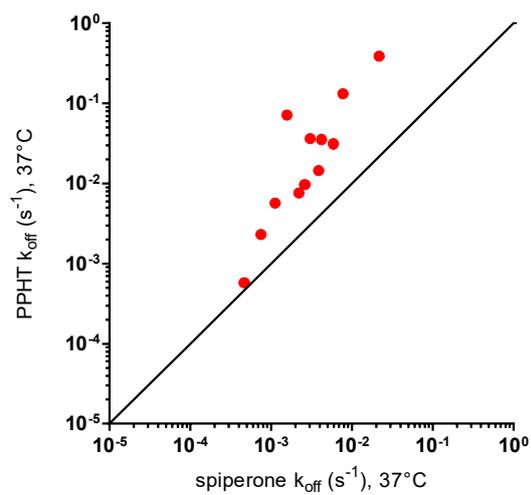
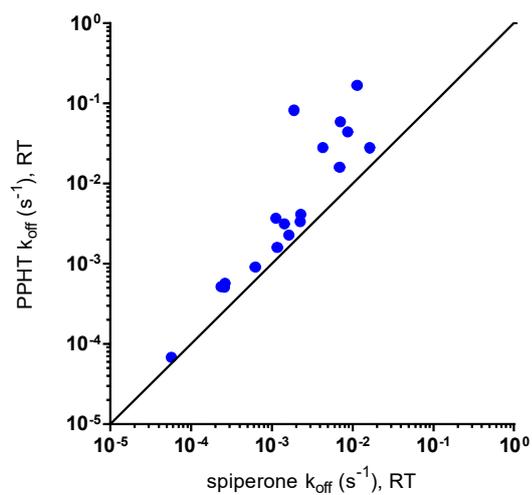
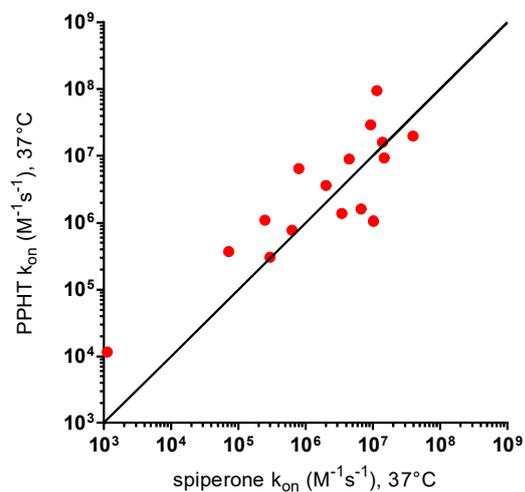
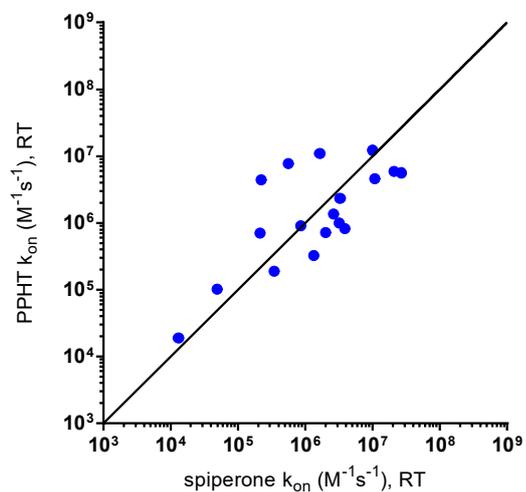
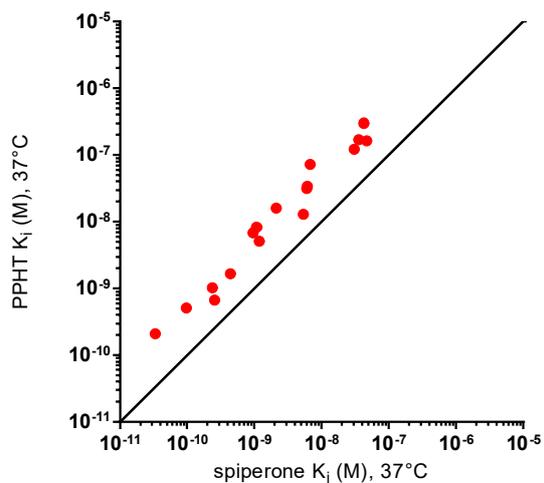
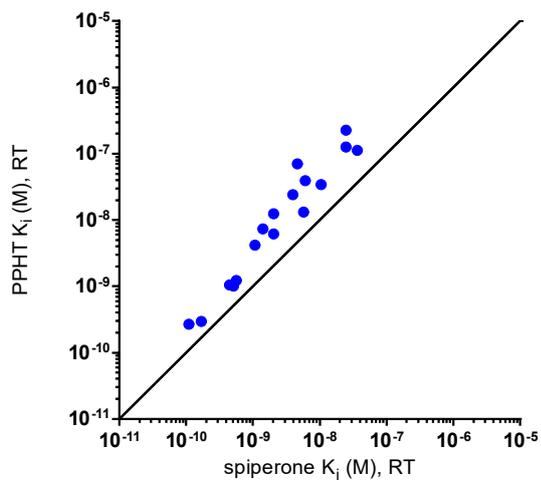


Figure S1g

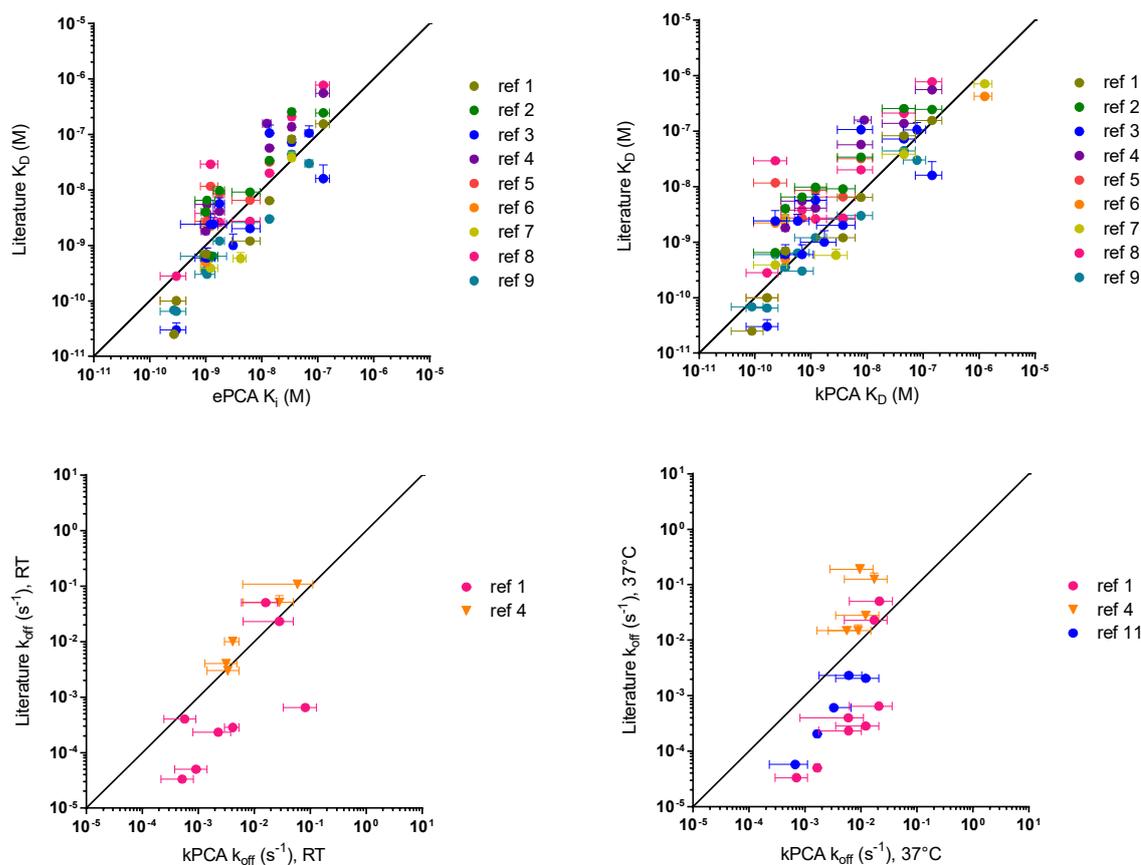


Figure S 1. Determination of affinity and kinetic parameters for the binding of Dopamine D_2 -receptor drugs using the TagLite[®] homogeneous time resolved fluorescence (HTRF) technology and the equilibrium and kinetic Probe Competition Assays (ePCA and kPCA). Symbols represent the measured data and lines the fits to the corresponding binding models. The compounds indicated with fastD2 and fastD2bu refer to JNJ-37822681 and JNJ-39269646, respectively.

a) Characterization of the PPHT tracer used in ePCA and kPCA at room temperature and at 37°C. The upper panel shows representative steady state titration curves, and the lower panel kinetic association- and dissociation curves at increasing tracer concentrations. HTRF signals were fit to the models specified in the methods section and the resulting binding parameters are indicated in the graphs. The data shown correspond to a single experiment with three replicates. Tracer input parameters used to compute the binding constants of test compounds were averaged from two independent experiments with three replicates each.

b-c) Representative kPCA traces (corresponding to a single experiment with two replicates) of the compounds listed in Table S1 at room temperature (b) and 37°C (c). Compound names are indicated on top of the graphs, Dosing is indicated by the color code specified on the right-hand side.

d-e) ePCA dose-response curves of the compounds listed in Table S1 at room temperature (d) and 37°C (e). Compound names are indicated on top of the graphs The different symbols represent different dilution series. Data shown represent the average of two independent experiment with two replicates each.

f) Comparison of the binding parameters obtained with PPHT-based tracer (agonist) and Spiperone-based tracer (antagonist).

g) Comparison of the binding parameters shown in Tables S1 and S2 with literature data. Reference numbers correspond to the following literature sources: 1 = (Kapur and Seeman, 2000), 2 = (Kroeze et al., 2003), 3 = (Burstein et al., 2005), 4 = (Langlois et al., 2012), 5 = (Kongsamut et al., 2002), 6 = (Toll et al., 1998), 7 = (Freedman et al., 1994), 8 = (Richelson and Souder, 2000), 9 = (Seeman and Tallerico, 1998), 11 = (Leysen and Gommeren, 1986),